Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Quaynor SD, Stradtman EW Jr, Kim H-G, et al. Delayed puberty and estrogen resistance in a woman with estrogen receptor α variant. N Engl J Med 2013;369:164-71. DOI: 10.1056/NEJMoa1303611

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Figure S1. A skeletal film of the left hand is shown. Note that the epiphyses are still open (bone density was read as normal on this film).

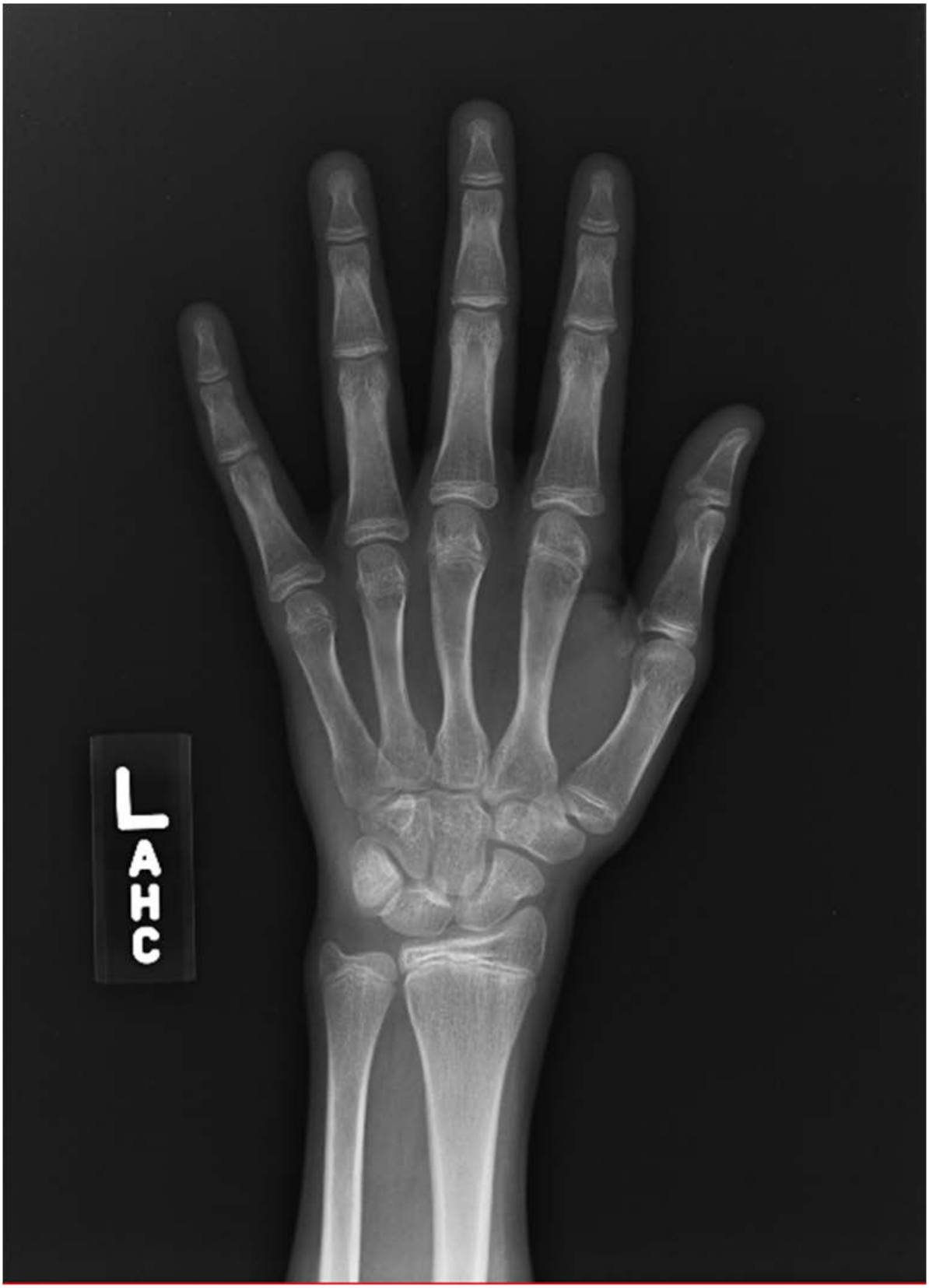

Figure S2. Western blot of whole cell lysates, using two different antibodies for ER-α—Millipore C1355 and Santa Cruz sc-532— shows the presence of the mutant receptor in both the mutant and WT in COS-7 cells. The molecular weight marker in kD is shown on the left.

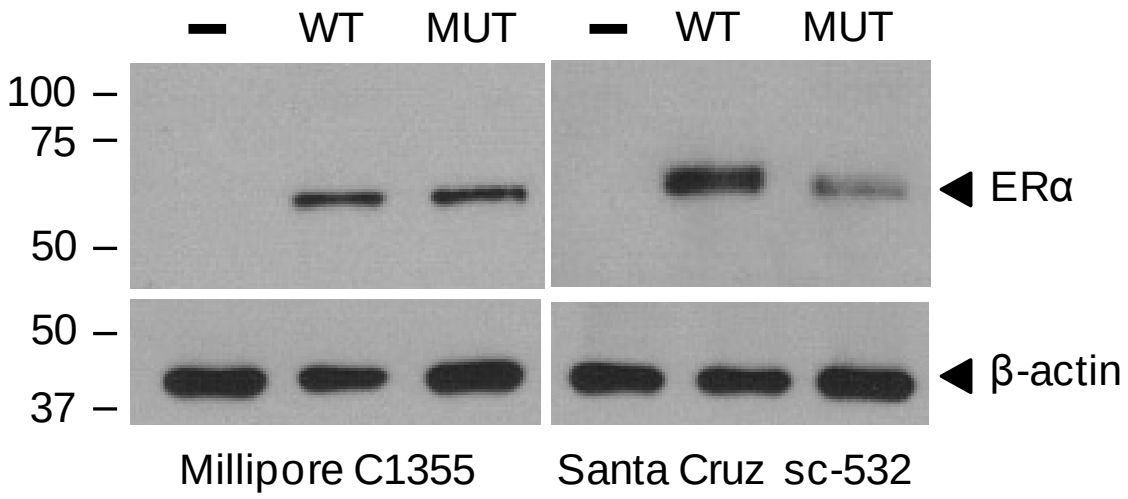

Figure S3. Immunofluorescence shows nuclear localization of both WT and mutant estrogen receptors in COS-7 cells transfected with either wild type or mutant ER-α. Arrows indicate the same nucleus for A and B (wild type); and for C and D (mutant). The ER-α antibody is shown on the left and DAPI staining is shown on the right.

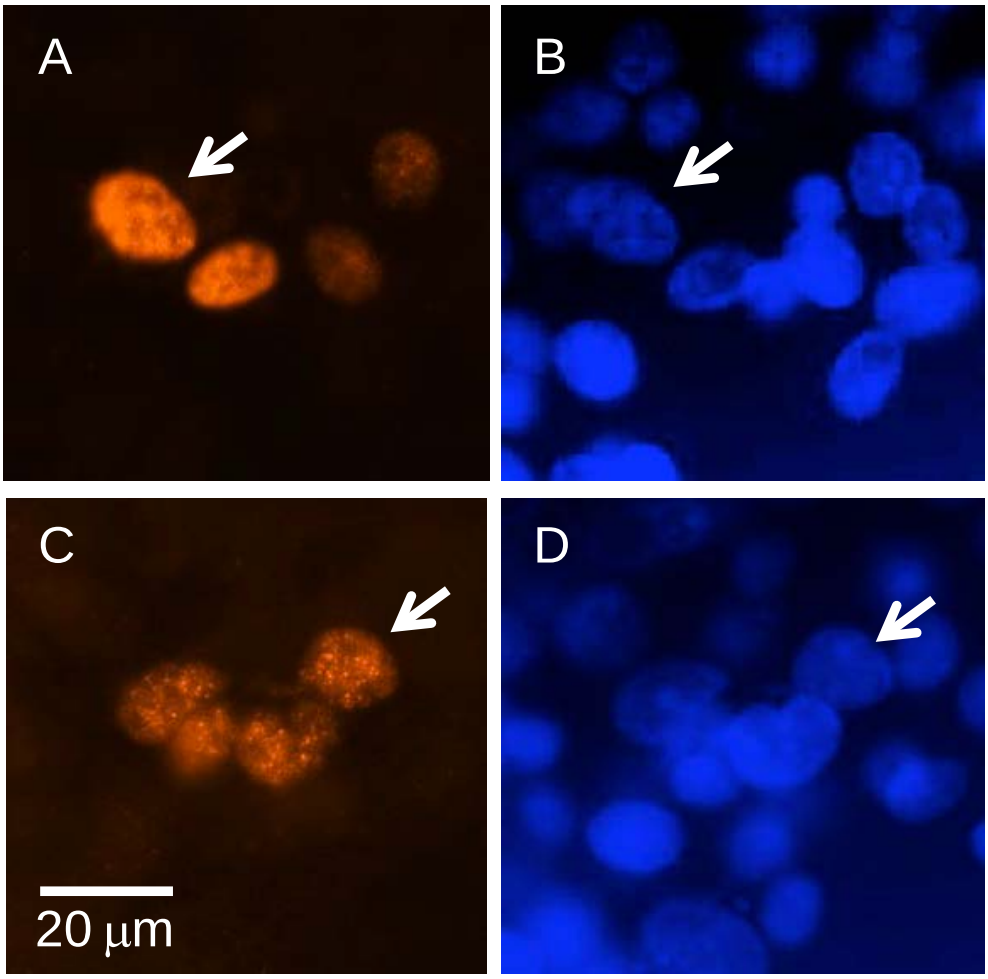

Table S1. Testing for Regions of Homozygosity.

An Affymetrix Cytoscan HD array which consisted of 750,000 SNP probes and 1.9 million additional copy number probes was used for detecting genome-wide homozygosity and copy number variants. 100 ng genomic DNA was labeled with Cytoscan reagent kit following manufacturer's recommendation, array data was analyzed with Chromosome Analysis Suite (ChAS) Software.

We detected nine large (>10Mb) ROH regions on eight chromosomes, strongly suggesting a parental blood relationship.[1,2] Supplemental Table 1 shows all ROH >3Mb. The total size of homozygosity is ~336Mb, which constitutes ~11.2% of the whole human genome using 3000Mb as the size of the whole genome (Affymetrix Cytoscan does not cover the entirety of the genome so the percentage of ROH may be somewhat higher than 11.2%). This level of homozygosity suggest a second degree relationship between proband's parents.[2]

Chr	Start	Stop	Size (KB)	Start	Stop	Marker Count
1	43,917,410	50,817,701	6,900	p34.2	p32.3	1,351
1	111,111,367	117,013,802	5,902	p13.3	p13.1	1,581
2	196,734,526	218,810,681	22,076	q32.3	q35	5,690
3	26,979,983	36,702,058	9,722	p24.1	p22.2	2,898
3	52,246,029	90,485,635	38,240	p21.2	p11.1	10,846
3	93,536,053	109,823,273	16,287	q11.1	q13.13	3,892
4	13,699,930	17,039,373	3,339	p15.33	p15.32	1,129
4	55,913,868	157,270,176	101,356	q12	q32.1	25,618
5	65,594,134	68,826,246	3,232	q12.3	q13.2	892
5	70,671,938	103,091,441	32,420	q13.2	q21.2	7,849
5	176,094,591	180,692,321	4,598	q35.2	q35.3	841
6	115,063,620	153,995,311	38,932	q22.1	q25.2	11,795
8	96,234,074	99,456,516	3,222	q22.1	q22.2	864
10	112,323,003	122,612,604	10,290	q25.2	q26.12	2,824
11	23,564,328	35,455,123	11,891	p14.3	p13	3,800
12	6,537,543	12,738,443	6,201	p13.31	p13.2	1,400
15	59,883,808	63,279,300	3,395	q22.2	q22.2	1,402
16	77,720,566	90,163,275	12,443	q23.1	q24.3	4,539
18	9,779,598	15,143,714	5,364	p11.22	p11.21	1,380
X	54,963,365	58,337,890	3,375	p11.21	p11.1	305

1. Papenhausen P, Schwartz S, Risheg H, et al. UPD detection using homozygosity profiling with a SNP genotyping microarray. Am J Med Genet A 2011;155A:757-68.
2. Sund KL, Zimmerman SL, Thomas C, et al. Regions of homozygosity identified by SNP microarray analysis aid in the diagnosis of autosomal recessive disease and incidentally detect parental blood relationships. Genet Med 2013;15:70-8.